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**NITROGEN DYNAMICS AND NITROGEN USE EFFICIENCY OF SPRING
CEREALS UNDER FINNISH GROWING CONDITIONS**

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ACADEMIC DISSERTATION

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ABSTRACT

Nitrogen (N) is one of the main inputs in cereal cultivation and as more than half of the arable land in Finland is used for cereal production, N has contributed substantially to agricultural pollution through fertilizer leaching and runoff. Based on this global phenomenon, the European Community has launched several directives to reduce agricultural emissions to the environment. Through such measures, and by using economic incentives, it is expected that northern European agricultural practices will, in the future, include reduced N fertilizer application rates. Reduced use of N fertilizer is likely to decrease both production costs and pollution, but could also result in reduced yields and quality if crops experience temporary N deficiency. Therefore, more efficient N use in cereal production, to minimize pollution risks and maximize farmer income, represents a current challenge for agronomic research in the northern growing areas.

The main objective of this study was to determine the differences in nitrogen use efficiency (NUE) among spring cereals grown in Finland. Additional aims were to characterize the multiple roles of NUE by analysing the extent of variation in NUE and its component traits among different cultivars, and to understand how other physiological traits, especially radiation use efficiency (RUE) and light interception, affect and interact with the main components of NUE and contribute to differences among cultivars.

This study included cultivars of barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.) and wheat (*Triticum aestivum* L.). Field experiments were conducted between 2001 and 2004 at Jokioinen, in Finland. To determine differences in NUE among cultivars and gauge the achievements of plant breeding in NUE, 17-18 cultivars of each of the three cereal species released between 1909 and 2002 were studied. Responses to nitrogen of landraces, old cultivars and modern cultivars of each cereal species were evaluated under two N regimes (0 and 90 kg N ha⁻¹).

Results of the study revealed that modern wheat, oat and barley cultivars had similar NUE values under Finnish growing conditions and only results from a wider range of cultivars indicated that wheat cultivars could have lower NUE than the other species. There was a clear relationship between nitrogen uptake efficiency (UPE) and NUE in all species whereas nitrogen utilization efficiency (UTE) had a strong positive relationship with NUE only for oat. UTE was clearly lower in wheat than in other species. Other traits related to N translocation indicated that wheat also had a lower harvest index, nitrogen harvest index and nitrogen remobilisation efficiency and therefore its N translocation efficiency was confirmed to be very low. On the basis of these results there appears to be potential and also a need for improvement in NUE. These results may help understand the underlying physiological differences in NUE and could help to identify alternative production options, such as the different roles that species can play in crop rotations designed to meet the demands of modern agricultural practices.

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LIST OF ORIGINAL PUBLICATIONS

The thesis consists of the following papers, which are referred to by their Roman numerals in the text.

- I Muurinen, S., Slafer, G.A. and Peltonen-Sainio, P. 2006. Breeding effects on nitrogen use efficiency of spring cereals under northern conditions. *Crop Science* 46: 561-568.

- II Muurinen, S., Kleemola, J. and Peltonen-Sainio, P. 2007. Accumulation and translocation of N in spring cereal cultivars differing in NUE. *Agronomy Journal* 99: 441-449.

- III Muurinen, S. and Peltonen-Sainio, P. 2006. Radiation-use efficiency of modern and old spring cereal cultivars and its response to nitrogen in northern growing conditions. *Field Crops Research* 96: 363-373.

- IV Muurinen, S., Hakala, K. and Peltonen-Sainio, P. 2007. Rubisco and nitrogen use efficiency of spring barley cultivars in northern growing conditions. *Manuscript*.

LIST OF ABBREVIATIONS

ANR	apparent nitrogen recovery
BPE	biomass production efficiency
f_i	radiation interception (%)
GLAI	green leaf area
HI	harvest index
k	light extinction coefficient
LMR	leaf mass ratio
NA	nitrogen uptake after anthesis
NHI	nitrogen harvest index
NRE	nitrogen remobilisation efficiency
NUE	nitrogen use efficiency
PAR	photosynthetically active radiation
P_n	leaf net photosynthesis
PNUE	photosynthesis nitrogen use efficiency
$\sum R_i$	estimation of accumulated intercepted radiation
RUE	radiation use efficiency
SA	specific activity
UPE	nitrogen uptake efficiency
UTE	nitrogen utilization efficiency
ZGS	Zadoks growth stage

1 INTRODUCTION

1.1 History and trends in N fertilizer use

Nitrogen occurs in many forms in the biosphere. The nitrogen content of the atmosphere is about 78%, but is generally not directly available to living organisms. Cereals lack symbiotic N₂ fixation and therefore require N to be added to the soil in the form of fertilizer. Before the 1920s some mining products were used as fertilizers in crop production in addition to organic sources such as guano (Lawlor et al., 2001). Industrial N fixation, the Haber-Bosch process, introduced in the 1920s, allowed N fertilizer to be produced from atmospheric N. This represented a starting point for the manufacture of numerous industrial and agricultural products. However, the turning point for N fertilizer use was the Green Revolution. The increase in yield potential brought about through new technologies, high yielding cultivars, irrigation and water management, plant protection agents and other farm management practices, was possible only with adequate application of fertilizers (Clark, 1990; Bumb, 1995). Because the availability of nutrients in the soil and from organic sources is generally inadequate, N is frequently the most used and also the most costly mineral nutrient required for cereal production (Clark, 1990). Globally, N is also considered to be the second most limiting factor for plant growth after water. Nitrogen and water availability interact strongly with each other in crop development (Sadras, 2005).

Since most fertilizers were easily available and relatively inexpensive after the 1960s, the improvement of soil nutrient status to meet the needs of plant mineral nutrition was the main objective of crop production, whereas plant mineral nutrient use efficiencies were neglected. The increase in fertilizer price following the oil crisis in the 1970s resulted in increased interest in plant mineral nutrient use efficiencies. Currently there exists concern about the effects of nitrate and nitrite on human health through consumption of food crops and about increased environmental problems associated with runoff and leaching of fertilizers. Maintaining agricultural production while limiting its adverse effects on the environment represents a major challenge that might be addressed through increased nitrogen use efficiency.

1.2 Impact of N fertilizer use on the environment

Intensive agricultural systems demand sufficient amounts of nutrients. The turnover of these nutrients and nutrient containing materials within agricultural systems far exceeds that in natural ecosystems (Steën, 2001). The effective use of nutrients in agricultural production systems has been addressed for several decades. Increased crop productivity has steadily raised N emissions to the environment, especially in the Netherlands (van Alphen and Stoorvogel, 2000). In Finland the risk of N leaching is more dependent on soil type and growing conditions (Pietola et al., 1999). Pietola et al. (1999) indicated that the risk of leaching is significantly higher after dry and warm late seasons since crop N uptake is reduced and surplus N is exposed to late season rains. The major concern is the negative

effects of N on the quality of surface and groundwater. These quality reductions are to a large extent considered to be attributable to agricultural source non-point nutrient pollution, which has been observed in many countries (Hatfield and Prueger, 2004; Torstensson et al. 2006). The problem is particularly recognizable in cool and humid regions, where large volumes of water percolate through soils during periods when there is no crop cover (Morecroft et al., 2000). Furthermore, Granstedt (2000) indicated that in the northern part of Europe the local and regional specialization of farms is an important reason for the high losses of plant nutrients. Some farm types specialize in crop production using artificial fertilizers, while others specialize in animal production with a high input of purchased animal feed and surplus plant nutrients in the form of manure, or even with additional artificial fertilizers. Therefore, this export of plant nutrients to the intensive farms makes for higher accumulation of manure, urine and fertilizers at single site and thus also increases the risk of nutrient losses to the environment (Granstedt, 2000).

Soil N can leave the agro-ecosystem through denitrification, volatilization and leaching (Raun and Johnson 1999; Cassman et al. 2003). Also, soil types differ in susceptibility to N losses. Soil denitrification is higher on clay soils, typical of many of the main cereal production regions of Finland, than on sandy soils, whereas the proportion of N lost through leaching is greater from sandy soils compared with clay soils (Granstedt, 2000).

1.3 N use regulations in Europe with reference to Finland

The soil N cycle is complex and involves many N transformations in a system of processes and reactions among the various soil N pools (Henriksen and Breland, 1999). Parameters including plant-available soil water content, temperature, organic matter content, history of organic manure use, crop rotation and return of crop residues, soil incorporation methods, among other factors, all impact on the turnover of N (Pietola et al., 1999; Lemola et al., 2000; Korsaaeth et al., 2002; Jensen et al., 2005). The complexity of the N cycle precludes accurate estimation of crop performance, yield and quality, and makes it challenging to estimate crop N use at the beginning of the growing season. Particularly in Finland, the common practice is to apply all fertilizers at sowing. N use recommendations for crop production are generally based on research and development work. The primary aim of the recommendations is to achieve the best financial return for the farmer by maximising the economic return from the fertilizer use while minimising the risk of N loss and the detrimental impact of nutrient use on the environment (Steèn, 2001).

Given that agriculture is considered to be one of the main sources of N emissions to the environment, the European Community launched the Agri-Environmental legislation and the Nitrates Directive to reduce water pollution caused or induced by nitrates from agricultural sources (EC-Council Directive, 1991). Therefore, in several northern European countries ceilings and limitations based on this directive have been taken into consideration when determining N fertilizer recommendations (Steèn, 2001).

The mean wheat yields and mean fertilizer application rates in Finland from 1986 to 2005 are presented in Fig.1 (Maatilatilastollinen Vuosikirja, 2005). The figure shows a reduction in mean N input in 1995 and some reduction in wheat yields immediately afterwards. Reductions in yields are likely explained by both dry growing seasons and reduction in fertilizer application rates and use of other inputs. However, the interest in new methods for producing crops using sustainable crop management practices has increased in northern Europe. Among such changes in agricultural systems have been organic farming practices, no-till cultivation and utilization of green manure crops, assuming they conserve essential nutrients and reduce the adverse impact of agriculture on water quality. A shift towards organic farming has received considerable political attention around the world (Trewavas, 2004). The Swedish government, for example, has set the goal that by the year 2010, 20% of agricultural land should be under organic farming, while currently it is about 7% (Torstensson et al., 2006). Finland is also close to the EU mean, with 6.6% of agricultural land under organic farming (Tietohaarukka, 2006) and the Finnish government has set the goal at 15% by 2010 (Lötjönen et al., 2004).

Torstensson et al. (2006) observed during a six year experiment that leaching of N was not reduced in organic systems, and the authors primarily attributed this to poor synchrony between the release of mineral N from the manure and the uptake of N by the crop. This also reduced the organically farmed crop yields by 20 to 80% compared with the same crop grown in conventional farming (Torstensson et al., 2006). In no-till systems N applied late is less available for mineralization than when plough-tillage is used (Kristensen et al., 2000). It has also been recognised in Finnish no-till experiments with cereals that obtaining high quality yields, similar to ordinary plough-till yields, no-till requires higher N fertilizer application rates (Käнкänen, personal communication 2006). When using green manure undersown, the N mineralization from crop material can be regulated by timing of incorporation into soil (Käнкänen et al., 1998, Lemola et al., 2000).

The official fertilizer recommendations in Finland are based on soil conditions, climatic zone, crop species and yield forecasts. However, after joining the EU in 1995, the fertilizer recommendations were applied according to the Agri-Environmental support programme. These regulations contain intermediate base limits for N application to all major crops. The farmers may apply more if there is reasonable evidence for high yields and high quality production, and providing the requirement of the Nitrate Directive is met, i.e. a maximum total application of 170 kg N ha⁻¹ contained in inorganic and organic fertilizer. Finland is the only country in the European Union that includes both inorganic and organic sources in the ceiling for N applications as stated by the Nitrates Directive. According to a Decision-in-Principle by the Finnish government in 1998, N loading from agriculture should be reduced by 50% by 2005 from the level at the beginning of the 1990s (Ministry of the Environment, 1998). It has been estimated that a 25-40% reduction in nutrient losses may be achieved by full implementation of the Finnish Agri-Environmental support programme (Valpasvuo-Jaatinen et al., 1997). However, there is no clear evidence yet that N losses are substantially

reduced by introduction and adoption of regulated N fertilizer use (Grönroos et al. 1998). The estimated total N load from agricultural land in Finland is 20 000 – 40 000 Mg y⁻¹ (Granlund et al., 2000). The average N leaching from spring cereal cultivation in Finland is about 10-35 kg ha⁻¹ y⁻¹ (Rekolainen et al. 1995; Lemola et al. 2000). Granlund et al. (2000) stated that the general goals for N load reductions will not be fully achieved by the reductions in N inputs and other management practices that have taken place during the first years of the Agri-Environmental support programme. In order to achieve further reductions, fertilizer and manure spreading should be reduced and better adjustment to the actual N requirement for crops should be further emphasized (Granlund et al., 2000). However, these N ceilings and limitations related to environmental concern are below the optimal economic rates on some farms.

1.4 Special emphasis on improved N use efficiency in Finland

Besides fertilizer management the recent markedly increased interest for field produced bioenergy has set new challenges for N use efficiency (NUE) in crop production. Reed canary grass (*Phalaris arundinacea* L.) is one of the new farm crops in Finland, and its role in large-scale energy production started in Finland in the late 1990s (Pahkala et al., 2005). The growth rate of reed canary grass is very slow after establishment (Pahkala et al., 2005) and this increases the risk of nutrient leaching. Barley-based ethanol and biodiesel production from turnip rape (*Brassica rapa* L.) do not necessarily reduce the greenhouse gas emission compared with fossil fuels (Mäkinen et al., 2006). The fertilizer use on these crops, as well as on other cereal crops, causes emission of nitrous oxide to the atmosphere. These emissions increase the amount of greenhouse gasses. Therefore, by increasing the yields and NUE of these biofuel crops the overall energy input per unit output ratio could be decreased.

Climate change is likely to result in slightly increased total runoffs in Finland (Bourauï et al., 2004). Adaptation of the Finnish agri-food sector to climate change will be further evaluated in a newly launched project as part of a national programme on adaptation to climate change (ILMASOPU, 2006-2009). The project concentrates on the link between climate change in the northern regions and production potential, market scenarios and associated risks. Therefore, an understanding of the processes that determine NUE in crops, particularly N uptake and distribution are important. This is particularly the case for spring barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.) and wheat (*Triticum aestivum* L.), as these are important crops and cover some 50% of the cultivated area in Finland. Furthermore, the increased N fertilizer price, compared with the low price of grain represents an economic concern for farmers. Therefore, the challenge is to link quality crop production efficiency with maintenance of environmental quality.

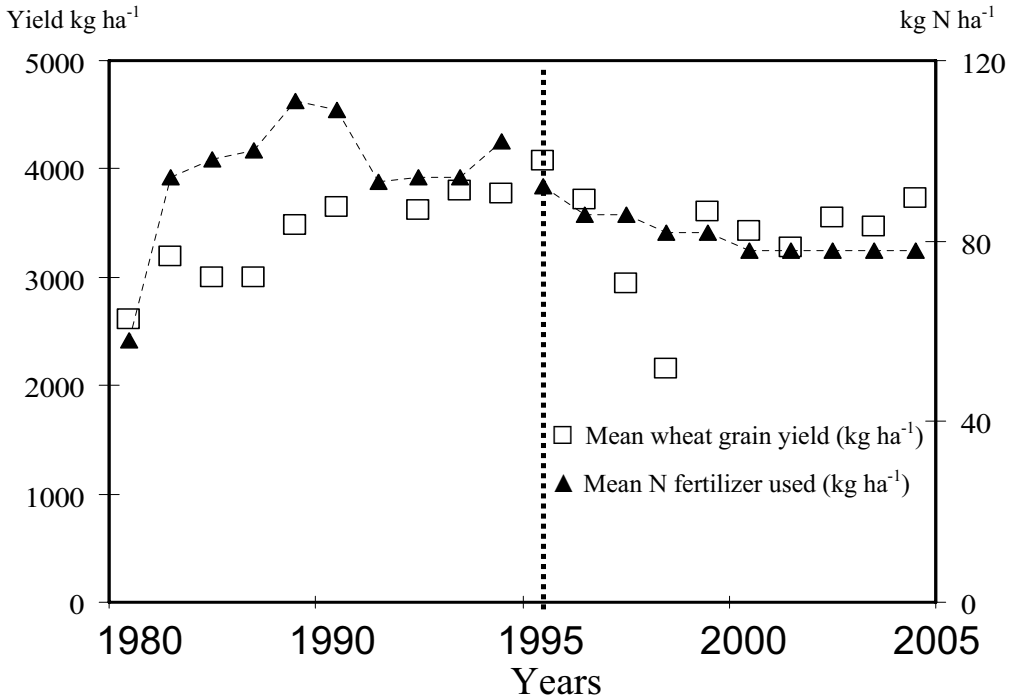


Figure.1. National mean wheat grain yield kg ha⁻¹ and average applied N fertilizer rate kg N ha⁻¹ from 1980 to 2005 in Finland (Maatilatilastollinen Vuosikirja, 2005; FAOSTAT, 2006).

1.5 Parameters for N use determination

Contrary to trends in Finland (e.g. Fig.1), global N fertilizer usage in cereal production has risen markedly. During the same period, grain yields have also risen. However, the amount of grain produced per unit of N applied has decreased (Hatfield and Prueger, 2004). Raun and Johnson (1999) calculated global N use efficiency:

$$NUE (\%) = \left[\frac{(total\ cereal\ N\ removed) - (N\ coming\ from\ the\ soil + N\ deposited\ in\ the\ rainfall)}{fertilizer\ N\ applied\ to\ cereals} \right] \times 100$$

For cereal production this is approximately 33%. Field experiments have indicated that no more than about 45-70% of the applied N fertilizers are recovered under average growing conditions (King et al. 2001; Noulas et al. 2004).

This is similar to the agronomic efficiency, the amount of economic yield (grain) per unit of N applied. Agronomic efficiency is the interaction of many N processes and reflects the overall efficiency with which applied N is used (Clark, 1990). It is based more on grain yield than on total dry matter yield. However, NUE is a complex trait. Moll et al. (1982) and Ortiz-Monastero et al. (1997) showed that NUE comprises N uptake efficiency (UPE) and N utilization efficiency (UTE). UPE reflects the efficiency of the crop in obtaining N from the soil, while UTE reflects the efficiency with which crops utilize N in the plant for the synthesis

of grain yield. In this way the contribution due to variations in N uptake could be separated from grain yield variations (Moll et al., 1982).

The calculation of the ability of a plant to extract N from the soil depends on whether or not residual soil N has been measured. Apparent N recovery (ANR), which is N in biomass in the presence of N fertilizer minus N in biomass with no N applied, is normally based on measurements of N uptake in the aerial plant biomass and depends on the assumption that fertilized and control plants absorb the same amount of soil N (Craswell and Godwin, 1984). In contrast, UPE, calculated as the total above-ground N per unit of N supplied, either includes available N from soil or not. Therefore, organic matter N mineralization plays an important role in the calculation of N uptake from the soil (Le Gouis et al., 2000). However, Youngquist et al. (1992) suggested that when initial soil nitrogen contents are equal, genotypic differences in UPE can be determined by measuring only plant N.

Moll et al. (1982) speculated that the contribution of UPE, (total amount of N taken up in plant material per unit of N supplied to the soil) to NUE increases with increasing soil N supply. However Ortiz-Monasterio et al. (1997) reported that UPE is an important component of NUE under low N conditions. For wheat, UPE accounts for most of the variation in NUE at low N availability (Ortiz-Monasterio et al., 1997; Le Gouis et al., 2000). Under northern growing conditions spring cereals are characterized by a rapid growth rate during a very limited period and therefore the ability to take up large amounts of N between germination and maturity is essential (Løes, 2003).

Average reported UPE values under fertilization in temperate regions for wheat and barley varied from 20% to 100% (Craswell and Godwin, 1984; Gauer et al., 1992; Le Gouis et al., 2000; Sinebo et al., 2003; Noulas et al., 2004). Results from experiments have shown genetic variation in cereals for UPE (Kelly et al., 1995; Singh and Arora, 2001). However, the correlation between total N uptake and year of cultivar release was weak (Slafer et al., 1990; Calderini et al., 1995; Foulkes et al., 1998), although a positive trend was reported for six-row spring barley (Bulman et al., 1993) and oat (Wych and Stuthman, 1983; Welch and Leggett, 1997). There is clear evidence that modern cereal cultivars produce higher yields when grown with conventional applications of fertilizer (Evans and Fischer, 1999) and it seems that the genetic gain has been especially large after 1960 (Austin, 1999). However, it is less clear whether modern cultivars perform better under conditions of limited N availability. Foulkes et al. (1998) found that the most recent cultivars were adapted to higher levels of N and took up relatively more N from fertilizer, whereas the older cultivars had relatively large uptake of N mineralized from soil. The N uptake from fertilizer was calculated as the apparent recovery of fertilizer N, which was N in yield with N fertilizer minus N in yield with no N applied. Feil (1992) indicated that cultivars producing large amounts of biomass seemed to have a more efficient nutrient uptake, which could decrease the total NUE of modern cultivars. Since N concentration is higher in leaves than in stems and sheaths N uptake may be more closely related to leafiness than to total shoot biomass (Feil, 1997).

On the other hand UTE, which is the ability to produce dry mass and thereafter grain yield from each unit of nutrient taken up, is expressed as the ratio of grain yield to total plant N (Moll et al., 1982; Cox et al., 1985). Results from experiments have shown genetic variation in cereals for UTE (Woodend et al., 1986; Papakosta, 1994; Singh and Arora, 2001) depending on genotype height. A strong relationship between UTE and harvest index (HI) was reported by Fischer and Wall (1976) who concluded that increased grain yield and HI also increased UTE. Ortiz-Monasterio et al. (1997) further subdivided UTE into HI and biomass production efficiency (BPE), affirming that HI has been most associated with UTE. Therefore, UTE appears to be the trait mainly affected by plant breeding (Slafer et al., 1990; Ortiz-Monasterio et al., 1997). HI has increased substantially through plant breeding (Austin et al., 1980; Bulman et al., 1993), whereas total shoot biomass has remained more or less unchanged during breeding history. Selection for higher HI may reduce the N storage capacity of the shoot (Feil, 1997). Therefore, the disadvantage of high UTE is that the nutrient value of the grain may be reduced, since reduction in protein N has been associated with high UTE (Ortiz-Monasterio et al., 1997; Calderini et al., 1995). Besides the better expression of UTE under high soil N (Ortiz-Monasterio et al., 1997) or under low soil N (Dhugga and Waines, 1989), there are indications of the differences between the cereal species in their NUE to UTE relationship. The association is especially strong for oat, regardless of N supply (Isfan, 1993). Furthermore, Delogu et al. (1998) showed that in low N input environments, winter barley had higher UTE than winter wheat and UTE was also associated with higher N harvest index (NHI), which is expressed as the proportion of the total N yield contained in the grain at maturity (Cregan and van Berkum, 1984). This indicates retranslocation efficiency of N from vegetative plant parts, i.e. not including roots, to the grain. Many studies have indicated that 70% or more of the N harvested in seeds is derived from N remobilized from senescing vegetative plant parts (Austin et al., 1977; Cox et al., 1985; Papakosta and Gagianas, 1991). Values of NHI are strongly affected by N fertilizer application (Ugalde, 1993) and climate (Feil, 1997). Even though there are differences among the species, the cereal NHI values are usually high, reaching and even exceeding 80% (Spiertz and de Vos, 1983; Ugalde, 1993; Feil, 1997; Noulas et al., 2004). Therefore, Cregan and van Berkum (1984) suggested that the more detailed trait to measure the ability of a crop to remove N from the vegetative parts would be N remobilisation efficiency (NRE). N remobilisation efficiency is therefore estimated as the fraction of stored N at flowering that is not recovered in the vegetative parts at maturity (Barbottin et al., 2005). Further, the improved understanding of plant N requirements and dynamics, particularly BPE and NRE from vegetative parts, among species and cultivars is needed to determine NUE better.

1.6 Objectives of this study

The purpose of this study was to increase understanding of N dynamics and N use efficiency of spring cereals under northern growing conditions with particular regard to increased concern about N leaching to the environment, drastic changes in crop management practices and increased need for sustainable bioenergy production. Since the growing season in Finland

is short and crop growth is intensive compared with that in other areas of cereal production, adaptation to these exceptional conditions has resulted in differences in dynamics of development and growth that are likely associated with differences in NUE. This requires specific breeding strategies for crop cultivars adapted to northern growing conditions. Information on N use and general characteristics of NUE of spring wheat, barley and oat are scarce, despite N nutrition itself having been a common subject for research in such regions.

The first objective was to determine the differences in NUE among spring cereals grown in Finland (I-II), with a subsequent objective of determining how plant breeding has affected NUE and its components (I). The third objective was characterization of the multiple roles of NUE, by analysing the extent of variation in NUE and its component traits among different cultivars. When having secured such comprehensive information, the last objective was to understand how other physiological traits, especially RUE, light interception and Rubisco content (III-IV) affect and interact with the main components of NUE, and how they contribute to the recorded differences among cultivars (II-IV). In these studies, barley was used as a model plant. A schematic presentation of how this study analysed NUE, its components and other traits relating to it, is shown in Fig.2.

The main working hypotheses tested were:

- 1) Modern spring cereals grown in Finland differ from each other in NUE due to their general differences in dynamics of development and growth, which are expected to be altered by management practices and breeding efforts.
- 2) As plant breeding has increased yield potential of spring cultivars markedly in Finland, the NUE of modern cultivars is indirectly increased when compared with that of old cultivars and landraces.
- 3) Under the particular Finnish growing conditions, the importance of NUE components and their interrelation differ from those reported in the literature.
- 4) N use of modern and old spring cereal cultivars is associated with radiation use efficiency and therefore biomass accumulation.

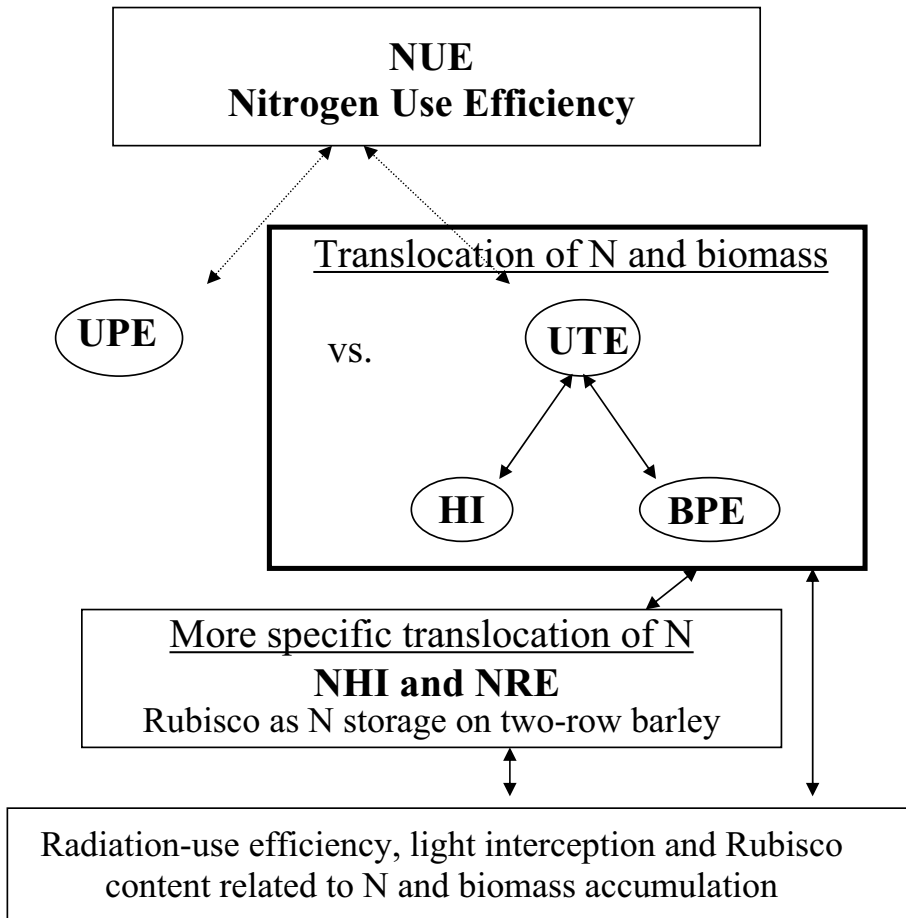


Figure 2. Schematic representation of how this work progressed in analysing nitrogen use efficiency (NUE), its components and other traits relating to it. The main components of NUE are nitrogen uptake efficiency (UPE) and nitrogen utilization efficiency (UTE). Traits relating to N translocation are harvest index (HI) and biomass production efficiency (BPE). Traits illustrating further detailed N translocation are nitrogen harvest index (NHI) and nitrogen remobilization efficiency (NRE).

2 MATERIAL AND METHODS

The experimental part of the work is described here in general outline. More detailed information can be found in the original publications (I-IV).

2.1 Plant material and experimental design

All experiments were conducted at MTT Agrifood Research Jokioinen, Finland. The first experiment, reported in papers II-IV, was conducted during the growing seasons of 2001-2003 on different fields. The experiments were arranged as split-plot designs with four replicates. Two N fertilizer application rates, 0 kg N ha⁻¹ and 90 kg N ha⁻¹, as ammonium nitrate, were applied to main plots at sowing. The plant material consisted of spring wheat, barley and oat cultivars (Table 1). Cultivar selection was based on differences in year of release (one landrace and two modern cultivars). The second set of experiments, reported in paper I, was conducted during the 2003-2004 growing seasons. The plant material included 17 or 18 cultivars of spring wheat, two-row barley and oat released between 1901 and 2002. The cultivars were sown in randomized complete block designs with three replicates. Main plots were period of release of cultivars and the three species were randomly assigned to subplots.

2.2. Dry weight and leaf area

Plants were uprooted for dry weight measurements. Plant samples from the first experiment were collected seven to eight times during the growing season at intervals of 7-14 days. Main shoots and tillers were separated and divided further into leaves, stems and heads. Samples were dried at 60°C for two days and weighed. Dry weight results from the first experiment were reported in paper III and used in calculations of other traits (Table 2) in papers II and IV. Dry weight results from the second experiment were used in calculations of traits for paper I. Green leaf area was measured separately for main shoots and tillers. Green leaf area index was obtained by multiplying the green leaf area per plant by the number of plants m⁻² for paper III.

2.3 Nitrogen concentration

Dried tissue samples were ground and N concentration for the various plant parts from the first experiment were determined using a Leco automatic analysing system (II). A Kjeldahl procedure with a Kjeltec Auto 1030 Analyzer was used in the second experiment (I). Plant part N content was calculated by multiplying dry matter by the N percentage.

Table 1. Crops, cultivars, N treatments and traits measured in experiments reported in the original papers.

Paper no.	Exp. year	Crop	Cultivars (or number of)	N kg ha ⁻¹	Measurements	Calculated
I	2003	Oat	18	70	Biomass N content	NUE
		Two-row barley	17			UPE
		Wheat	18			UTE
	2004	Oat			HI	
		Two-row barley			NHI	
		Wheat			BPE	
II	2001	Oat		0	Yield Biomass N content	NA
		Two-row barley				NUE
		Six-row barley				UPE
		Wheat				UTE
	2002	Oat	Aslak, Suomi, Jama	and	HI	
		Two-row barley	Inari, Scarlett, Uurainen	90	NHI	
		Six-row barley	Kunnari, Rolf, Olli		BPE	
	2003	Wheat	Manu, Vinjett, Tammi		NRE	
		Oat				
		Two-row barley				
Six-row barley						
III	2002	Oat	Aslak, Suomi, Jama	0	Biomass GLAI PAR	$\sum R_i$
		Two-row barley	Inari, Scarlett, Uurainen			k
		Six-row barley	Kunnari, Rolf, Olli			RUE
		Wheat	Manu, Vinjett, Tammi			
	2003	Oat		90		
		Two-row barley				
IV	2002	Two-row barley	Inari, Scarlett, Uurainen	0	photosynthesis	
					init. act. Rub.	PNUE
	2003	Two-row barley			and	LMR
					tot. act. Rub.	SA
				90	Rub. content	
					Prot. content	

BPE, biomass production efficiency; GLAI, green leaf area; HI, harvest index; JAMA, cultivar Jalostettu maatiainen; k , light extinction coefficient; LMR, leaf mass ratio; NA, nitrogen uptake after anthesis; NHI, nitrogen harvest index; NRE, nitrogen remobilisation efficiency; NUE, nitrogen use efficiency; PAR, photosynthetically active radiation; PNUE, photosynthesis nitrogen use efficiency; Rub., Rubisco; $\sum R_i$, estimation of accumulated intercepted radiation; RUE, radiation use efficiency; SA, specific activity; UPE, nitrogen uptake efficiency, UTE nitrogen utilization efficiency

2.4 Light measurements

Light interception measurements were done in the first experiment to calculate the radiation-use efficiency for paper III. Photosynthetically active radiation was measured above and beneath canopies using the Sun Scan Canopy Analysis System. As the lowermost leaves turned yellowish, the light measurements were performed just above the yellowing leaves. The results were used for further calculations of fractional PAR interception. Measurements were taken at intervals of one week from approximately 34 days after planting (ZGS 13-14) to 77 days after planting (i.e., in the middle of the grain filling period). Measurements were taken on clear or slightly hazy days during the 2 h around solar noon, based on results from preliminary tests.

2.5 Photosynthesis and Rubisco activity

Flag leaf photosynthesis and Rubisco content and activity were measured for paper IV. In 2002, fully developed penultimate leaves were measured, as the flag leaves were too small for measuring due to severe drought. In 2003, fully developed flag leaves were measured. Measurements were taken at three growth stages. They were initiated after the beginning of heading (Zadoks growth stage ZGS 50-55, Zadoks et al., 1974) and terminated when grain filling was close to complete but upper canopy leaves were not fully senesced. Single leaf photosynthetic rates ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) were determined using a portable CO_2 measuring system, combined with a Parkinson Leaf Cuvette for narrow leaves (LCA-3, ADC Co., Hoddesdon, UK).

The leaf segments used to assess photosynthesis were collected for measurements of Rubisco activity ($\mu\text{mol m}^{-2} \text{ s}^{-1}$), and quantities of Rubisco protein and total soluble protein (g m^{-2}). Initial activity and total activity of Rubisco were measured spectrophotometrically by coupling CO_2 fixation to NADH oxidation, which was recorded as decline of absorbance at 340nm (Lilley and Walker 1974). Total soluble leaf protein was quantified using the simple protein-dye binding method of Bradford (1976), using BSA as a standard. The samples for the chlorophyll measurements were extracted in 80% acetone buffer with 25 mM Hepes-KOH (pH 7.5) and determined by the method of Porra et al. (1989).

The concentration of Rubisco protein in the crude extract was measured by separating the protein by SDS-PAGE with the discontinuous electrophoresis buffer system of Laemmli (1970). The absorbance of the SDS solution after extraction of dye was measured at 585 nm using the method of Bradford (1976).

2.6 Statistics

All statistical analyses were carried out using PROC MIXED (Littell et al., 1996) of the SAS Institute Inc. To compare cultivars the lsmeans statement of PROC MIXED was used to produce t-type tests (II-IV) and robust REGRESSION was used to analyze the trait changes

(I). Before all the analyses, equality of group variances and normality of error assumptions were checked using Box-Cox diagnostic plots (Neter et al., 1996).

Table 2. Traits measured and calculated from two experiment sets

Exp.	Trait		Explanations and calculations of the traits
1	Biomass	g plant ⁻¹	all plant parts separately
	plant N cont.	%	measured by Leco
	NUE	kg kg ⁻¹ N	multiplying UPE by UTE
	UPE	%	ratio of (tot. N 90-tot. N 0) to applied fertilizer amount
	UTE	kg kg ⁻¹ N	multiplying HI by BPE
	HI	%	ratio of grain yield to total above ground biomass
	BPE	kg kg ⁻¹ N	ratio of tot. above ground biomass to tot. above ground N
	NHI	%	ratio of grain N yield to total above ground N
	NA	mg	tot. aboveg. N at maturity minus tot. aboveg. N at anthesis
	NRE	%	ratio of (grain N - NA)to total above ground N at anthesis
	GLAI	m ² m ⁻²	multiplying leaf area by the number of plants m ⁻²
	<i>f_i</i>	%	ratio of radiation intercepted to incident rad. above canopy
	$\sum R_i$		accumulated intercepted radiation
	<i>k</i>		slope of regression of ln(1- <i>f_i</i>) on GLAI
	RUE	g MJ ⁻¹	slope of accumulation of above ground biomass against $\sum R_i$
	PNUE	μmol CO ₂ (mmol N) ⁻¹	ratio between P _n to leaf N content
	LMR	%	ratio of the leaf dry mass to the total plant dry mass
	Rub.in.act.	μmol CO ₂ assimilated m ⁻² s ⁻¹	
SA	μmol CO ₂ mg ⁻¹ Rubisco min ⁻¹	total Rubisco activity per unit of Rubisco protein	
Tot. soluble protein	g m ⁻²	measured spectrophotometrically	
Rub. protein	g m ⁻²	measured with electrophoresis	
Chl	g m ⁻²	measured spectrophotometrically	
2	Biomass	g plant ⁻¹	all plant parts separately
	plant N cont	%	measured by Kjeldahl procedure
	NUE	g g ⁻¹ N	ratio of grain yield to total available N
	UPE	%	ratio of tot. above ground N to tot. available N
	UTE	g g ⁻¹ N	ratio of grain yield to total above ground N
	HI	%	ratio of grain yield to total above ground biomass
	BPE	%	ratio of tot. above ground biomass to tot. above ground N
	NHI	g g ⁻¹ N	ratio of grain N yield to total aboveground N

BPE, biomass production efficiency; *f_i*, radiation interception; GLAI, green leaf area; HI, harvest index; *k*, light extinction coefficient; LMR, leaf mass ratio; NA, nitrogen uptake after anthesis; NHI, nitrogen harvest index; NRE, nitrogen remobilisation efficiency; NUE, nitrogen use efficiency; PAR, photosynthetically active radiation; PNUE, photosynthesis nitrogen use efficiency; $\sum R_i$, estimation of accumulated intercepted radiation; RUE, radiation use efficiency; SA, specific activity; UPE, nitrogen uptake efficiency, UTE nitrogen utilization efficiency

3 RESULTS AND DISCUSSION

The three cereal species differed in their N-use traits, indicating that there exists some further potential to improve NUE through crop management and plant breeding. Regarding N dynamics and especially NUE characteristics, information on spring cereals grown under northern European growing conditions is scarce and scattered. In our study, the NUE values for wheat averaged 29.4 g g⁻¹N in the second experiment (I) and 16.8 g g⁻¹N in the first experiment (II). The values from the second experiment (I) were within the range of 26-44 g g⁻¹N, as reported elsewhere (Ortiz-Monasterio et al., 1997). The earlier reported values for barley and oat largely represent agronomic efficiency of N use rather than NUE and vary widely depending on growing conditions (Isfan, 1993; Delogu et al., 1998; Sinebo et al., 2003). Despite this, as the values for barley averaged 32.8 g g⁻¹N (I) and 24.2 g g⁻¹N (II) and for oat 27.1 g g⁻¹N (I) and 26.3 g g⁻¹N (II), they were also close to the range of reported values for wheat. As no substantial differences in NUE were found among spring cereal species when comparing the results from three modern cultivars (I), this might indicate that past plant breeding has been impartially equal for all spring cereal species on N use traits under these growing conditions. There existed no consistent difference among modern spring cereal cultivars in NUE, but expression of differences in NUE performance was to some extent dependent on the year. For example, in 2001 and 2002 for the first experiment (II), results were more variable than between 2003 and 2004 in the second experiment (I). This finding and differences in cultivar selection used for mean NUE value calculation resulted in lack of consistency in results between the experiments. For example, the second experiment (I) included only three modern cultivars out of a total of eighteen cultivars, while the first experiment (II) included all three cultivars used in experiment i.e. one long-strawed landrace included.

The global cereal grain N use efficiency, which is measured as:

$$NUE (\%) = \left[\frac{(\text{total cereal N removed}) - (\text{N coming from the soil} + \text{N deposited in the rainfall})}{\text{fertilizer N applied to cereals}} \right] \times 100$$

Can be as low as 33% (Raun and Johnson, 1999), even though several studies show improved NUE in modern wheat cultivars (Austin et al., 1980; Slafer et al., 1990; Calderini et al. 1995; Ortiz-Monasterio et al. 1997; Foulkes et al. 1998; Reynolds et al. 1999) and improved agronomic efficiency of modern barley cultivars (Isfan 1990; Delogu et al. 1998; Abeledo et al., 2003) compared with their predecessors. Also in our study there were significant and positive linear relationships between NUE and year of cultivar release for wheat and oat, but not for barley (I). The genetic gain estimated for NUE was highest in oat (0.13 g N g⁻¹ y⁻¹) while the improvement achieved in wheat was intermediate (0.05 g N g⁻¹ y⁻¹). The genetic gains in NUE for wheat and oat were associated with improved yield potential and grain yields (Ortiz et al. 1998; Slafer and Peltonen-Sainio, 2001; Öfversten et al., 2004). It appears that the extent of NUE improvement during the subsequent breeding process under northern

growing conditions was dependent on the basis of NUE values of landraces and old cultivars at the beginning of the 20th century. Greater increase in NUE improvement of oat, compared with barley and wheat (I), and lower NUE values for old oat cultivars (I) could be explained by the different time periods of adaptation to the growing conditions. Based on our results, it seems that the NUE of barley landraces and old cultivars was initially higher compared with the NUE of wheat landraces and old cultivars of oat. The differences in initial NUE values could be explained by the fact that barley was the more common crop in Finland after the 14th century, whereas oat and wheat were minor crops at that time (Aikasalo, 1988; Rousi, 1997).

3.1 UPE and UTE contribution to NUE

There were no significant differences among the cereal species in UPE (I and II). On the other hand, the relationship between NUE and UPE was positive and consistent within each of the three species (I), indicating that UPE is an important component of NUE. This is in accordance with results reported by Moll et al. (1982) for high soil N conditions and Ortiz-Monasterio et al. (1997) for low soil N conditions. Even though the soil N availability varied in our study according to year, the NUE and UPE relationship was always positive (I). However, cultivars within species differed, indicating that low yielding cultivars had also lower UPE (II). Nevertheless, closer examination of the improvement in UPE through breeding did not indicate any consistent trend of increased N uptake for wheat and barley during the last century (I). This is in line with the study of Løes (2003) that showed similar nutrient uptake efficiency for old and modern cultivars. In contrast to these findings, Slafer et al. (1990), Calderini et al. (1995) and Foulkes et al. (1998) showed for wheat that the oldest genotypes released in the period 1969-1988 had a higher uptake of N from soil without additional N supply, whereas the most recently released genotypes had a relatively higher uptake of N from applied N fertilizer. A positive trend between total N uptake and year of cultivar release was also reported for six-row spring barley (Bulman et al. 1993) and oat (Wych and Stuthman 1983; Welch and Leggett 1997). In our studies calculations of UPE were done based on the soil N content in the second experiment (I) and difference in plant N contents between 0 and 90 kg N ha⁻¹ fertilizer application rates in the first experiment (II). These differences between the two calculation methods could account for inconsistency between the results and might bias the comparison. However, both methods of calculation somewhat underestimate the total N available, as mineralization takes place during the growing season and therefore UPE in our experiments could be considered more as an estimate than as an accurate value.

Differences between species in N uptake were characterized by referring to the N contents of the plant material at heading and maturity. Barley took up 70 to 73% of the total N before heading, whereas wheat and oat averaged 64% (II). Our results for barley are in line with those of earlier studies (Bulman and Smith, 1994; Delogu et al., 1998), while wheat had much lower N uptake up to heading than the 90 to 100% reported by Clarke et al. (1990) and Heitholt et al. (1990). According to our results, oat and wheat had up to 69% higher post-

heading N uptake than barley, possibly because they require much longer growing periods under northern growing conditions than barley (Juuti, 1988; Mukula and Rantanen, 1989; Peltonen-Sainio et al., 2007b). Therefore, our results do not fully agree with those of earlier studies which suggested that higher N uptake of wheat would contribute to improved NUE in wheat (Van Sanford and MacKown, 1986; May et al., 1991; Le Gouis et al., 2000).

Wheat had significantly lower UTE than the other species (I and II), demonstrating the ability of the crop to translocate the N taken up into the grains. UTE is considered to be an essential physiological parameter contributing to improved NUE (Isfan, 1993). However, only oat exhibited a consistent, positive relationship between NUE and UTE (I). Nevertheless, the fact that there were no differences were evident between species in UPE though the differences in timing of N accumulation (II), could indicate that UTE contributes to the differences in NUE recorded between the spring cereal species grown under northern growing conditions. The observed differences in UTE between species were consistent with earlier results showing that barley outperformed wheat regarding UTE (Delogu et al., 1998).

Wheat and oat showed genetic improvements in UTE (I), which is in line with the earlier results for Nordic spring wheat (Ortiz et al., 1998). However, there was no genetic gain in UTE for two-row barley (I), even though, in general, cultivars differed in UTE within all species (II). The only indication of plant breeding effects on barley was that modern six-row barley cultivars tended to have higher UTE in all three years at both N treatments (II). In contrast to these findings, Górný (2001) indicated that as N stress increased in barley, the UTE of landraces and old cultivars exceeded those of modern ones. Differences between these two experiments on UTE performance within modern and old barley cultivars could be because the second experiment (I) only contained the two-row barley cultivars and the first experiment (II) included the old two-row cultivar which differed greatly from other landraces and also from early 1920's cultivars in the second experiment (I) and emphasised the difference in NUE and its components.

The need for improvements in UPE to enhance further increases in NUE can not be underestimated for northern growing conditions. However, it seems that UTE in particular with both fast and efficient translocation of N in oat, wheat and six-row barley, could play a more important role in enhancing NUE in the future. The fast growth rate of six-row barley sets high demands for a steady N source during grain filling and low N accumulation before heading in oat and wheat sets high demands on post-heading N translocation.

3.2 HI and BPE contribution to UTE

Ortiz-Monasterio et al. (1997) defined UTE as a combination of HI and BPE and showed that new wheat cultivars had improved HI rather than improved BPE. Earlier studies on spring wheat and barley grown in the Nordic region showed that improvement in UTE was achieved through reduced plant height and lodging and enhanced yields via higher HI (Ortiz et al.,

1998, 2002). In our study, which included lines released at different time periods, UTE was positively correlated with HI for wheat and oat, but not for barley (I). Mean HI values calculated from the first experiment (II) indicated that wheat (37%) had lower HI than two- and six-row barley (49% and 55% respectively) and oat (50%) (unpublished data), similar to the report of Peltonen-Sainio et al. (2007b). These findings support the conclusion that low UTE of wheat is related to its low HI compared with the other species (II). On the other hand, Peltonen-Sainio et al. (2007b) indicated that HI of wheat could be further increased under Finnish growing conditions, since it was clearly below the maximum value of six-row barley. Hence, due to the positive correlation between UTE and HI, UTE in wheat could be concomitantly increased with improvements to HI. However, it seems that in barley and oat HI is close to its maximum and further improvements in grain yield and in UTE are expected through increasing the total above-ground plant biomass while maintaining the HI at the current level.

The lowest HI values typical for old oat and wheat cultivars (Urainen 41%, Jalostettu maatiainen 48%, Tammi 34% and Olli 50%) were not always related to UTE since, for example, modern cultivars Manu and Aslak had the lowest UTE values. This could indicate that the improvement of HI in new oat and wheat cultivars would not fully guarantee improved N utilisation and hence, biomass production efficiency should also be considered in addition to other traits related to N translocation, such as NRE.

Above-ground biomass accumulation was highest in oat (III) and ranged from 1200 g m⁻² in modern cultivars up to 1900 g m⁻² in the old landrace. Wheat had somewhat higher biomass accumulation compared with barley cultivars. Within species the cultivars accumulated less than half of their above-ground biomass prior to the heading. However, there were differences between the experiments in terms of biomass accumulation rate after heading (III). In 2002, some periods of drought likely affected the biomass accumulation after heading and biomass accumulation rate decreased or even ceased for some cultivars (III). BPE measures total plant biomass produced per unit N absorbed, demonstrates N concentration in the entire plant and describes the internal N requirement in species (Gourley et al., 1994). The correlation between BPE and UTE was not significant for any of the spring cereal species (I). In fact, there was actually a negative trend between UTE and BPE in wheat and oat (I). Further modified BPE_{vege} took into account above-ground vegetative biomass, that was divided by plant total above-ground N. BPE_{vege} was higher in wheat than in other species even at the end of the growing season. Hence, both UTE and HI were low in wheat compared with other species. These results suggest that in wheat there exists competition between vegetative and reproductive organs (II), or more precisely, wheat produces more biomass per unit of N uptake without efficient remobilisation into the grains. This is also supported by the findings that wheat yields were lower than those of the other species in each of the years compared (II), even though the biomass accumulation between species did not differ substantially (III). In addition, there were no significant differences between barley and oat until the end of the growing period, when six-row barley differed from all others and had lower BPE values (II).

This indicated that six- row barley did not produce biomass so efficiently with the N taken up, but instead transferred biomass to the grains, based on the high HI values.

Sinclair and Jamieson (2006) indicated that N can be an important limiting factor for yield by contributing to the determination of grain number through the development of the spikes and florets. Triboi and Triboi-Blondel (2002) proposed that if the N demand per plant is greater than the N uptake by roots during grain filling, the pre-stored N in the vegetative tissue is rapidly remobilized into the grains. This appears to be the case with spring barley in our study. However, the variable performance of the spring barley and wheat in biomass and N translocation might be explained by differences in their grain number and grain weight interaction. Peltonen-Sainio et al. (2007a) showed that spring barley had better ability to compensate for the reduced grain number by enhanced grain filling than spring wheat under northern growing conditions. This is also supported by the result from our study (II) showing that barley yields during the years of experimentation were closer to their long-term average than were wheat yields. Therefore, the low N accumulation recorded before heading of wheat (II) would decrease the floret set and potentially the grain number and, after heading, decrease UTE, and HI performance would be sink limited.

Differences in green leaf area index (GLAI) values between the species did not show wheat to differ significantly from the other species. This did not indicate greater biomass accumulation to the leaves (or leaf size) during the growing season (III). However, wheat accumulated N to the tillers at relatively high rates (II), which could support the earlier assumption about the competition for N between vegetative and reproductive organs. Even though the long days under northern growing conditions are reported to inhibit tillering and favour the main shoot dominance (Michael and Beringer, 1980), the tillering ability of the plants and tiller productivity were also emphasised between the spring cereal species (Peltonen-Sainio and Järvinen, 1995; Rajala and Peltonen-Sainio, 2002). The average number of head-bearing tillers was 0.28 for oat, 0.41 for wheat, 0.86 for two-row barley, and 0.30 for six-row barley (unpublished data). These numbers were close to values reported by Peltonen-Sainio et al. (2007b). The higher number for wheat in our study, compared with 0.31 reported by Peltonen-Sainio et al. (2007b) clearly resulted from the old cultivar Tammi. However, these numbers support the earlier assumption about the competition between the vegetative plant parts and grain filling in wheat, since wheat produces large total numbers of tillers and about 47% of them bear heads. Yet the actual grain number in tiller heads is low: for wheat six per plant whereas it is five for oat, 11 for two-row barley and nine for six-row barley (unpublished data).

3.3 Other traits characterising N accumulation and translocation

The proportion of the total plant N allocated to the grain at maturity, (NHI), was significantly lower in wheat than in oat and barley. Average NHI for barley (77%) and wheat (74%) (II), were close to the values cited previously (Spiertz and De Vos, 1983; Ugalde, 1993; Bulman

and Smith, 1994; Noulas et al., 2004). However, NHI for oat was, during all years, higher than the values of 69-75% reported by Rattunde and Frey (1986), McMullan et al. (1988) and Welch and Leggett (1997).

NRE, which represents a measure of the ability of the crop to remove N from vegetative tissues, was inconsistent for wheat and two-row barley over the three years (II). As the weather conditions during the grain filling in 2002 were dry, N movement was suppressed in the soil and this resulted in lower post-anthesis N uptake compared with that recorded in the 2001 and 2003 experiments. The decreased N availability during grain filling might also have increased NRE values for all cultivars of two-row barley and wheat during 2002. Wheat NRE varied markedly during the three years and its mean value was significantly lower compared with that of the other species (II). This result was consistent with the findings of Cox et al. (1985), Papakosta and Gagianas (1991) and Palta et al. (1994).

The results from different N treatments in this study did not support the assumption that lower N supply (0 kg N ha^{-1}) would result in a higher NRE than the 90 kg N ha^{-1} treatment. This might indicate that at higher rates of N availability, N does not need to be translocated from vegetative plant parts to fill grains as efficiently as in the case where N could not be taken up from the soil. In contrast to this hypothesis, 90 kg N ha^{-1} treatments resulted in higher NRE than 0 kg N ha^{-1} treatments in all three years for all crops. However, Barbottin et al. (2005) showed that in some cases for wheat, the NRE could be stable across the environments and genotypes. Our results for oat and six-row barley indicated stable mean NRE values within the years, suggesting that NRE for these crops might not be so controlled by the environment. Our NRE values for barley were 65-78% and they varied less than the reported 47-66% for spring barley (Przulj and Momcilovic, 2001). On the other hand, the mean values reported by Cregan and van Berkum (1984) and Barbottin et al. (2005) for wheat ranged from 66 to 92%, whereas our results for wheat ranged from 47% to 72% (II). Our results for oat were in line with those reported previously for barley and wheat (Przulj and Momcilovic, 2001).

A comparison of NHI and NRE in this study revealed that both were low for wheat, average for barley (both two- and six-row), and high for oat. Linking this with the recorded differences in post-anthesis N taken up suggests that in wheat the proportion of assimilated N used immediately in the developing grains is greater than in barley and oat. Also, the comparison between barley and oat indicated that barley had more efficient pre-heading assimilation processes and, above all, better translocation ability. Similar differences between species were reported by Cregan and van Berkum (1984), who compared wheat, soybean (*Glycine max* L.) and maize (*Zea mays* L.). Within a species there were indications that similar assimilation efficiency differences were apparent among cultivars. Wheat cultivar Vinjett and the oat landrace had lower NRE and higher NHI than other cultivars, suggesting that a large proportion of assimilated N is immediately used by the developing grains rather than first being incorporated into leaf or stem proteins, as is likely to be the case in other wheat and oat cultivars.

The correlation between NRE and leaf and tiller traits measured from mature plants indicated that NRE had a strong negative relationship between main shoot leaf and straw N content and tiller traits for barley and oat. This could indicate that barley removed more N from leaves and tillers at late grain filling, thereby, supporting evidence for there not being strong competition between vegetative plant parts and grain filling. Contrary to this, vegetative parts and grains of oat likely competed more for N at higher N treatments as there were no strong, negative correlations between NRE and tiller traits. This suggests that some of the tillers possibly competed with filling grains for N. However, wheat NRE correlated strongly and negatively with tiller traits in the main. This suggests that N translocation would have been from tillers during a very late grain-filling phase, whereas there was no strong N translocation from vegetative parts of the main shoots, which could still have been competing with filling grains for N.

3.4 N storages in leaves: case study for two-row barley

Two-row barley cultivars exhibited high levels of N translocation, based on values of NHI and NRE. The possible differences in N storage capability of the main shoot leaves were compared further by studying modern and old two-row barley cultivars. Since the modern cultivar Scarlett had higher NHI than the old cultivar Uurainen, Scarlett translocated more N to the grains than did Uurainen (II). However, the NRE did not differ between the cultivars (II). In 2002, there was a more rapid decrease in Rubisco protein content in Scarlett than in Inari and Uurainen. However, in 2003 Scarlett and Uurainen showed a similar decrease in Rubisco protein content of leaves (IV). The decrease in Rubisco protein content was associated with a decrease in leaf N (IV), which is in agreement with the findings of Pérez et al. (2005). The recorded decrease was most probably a consequence of reallocation of N within the plant rather than decrease in the total N uptake, since average N uptake after anthesis was positive for all three cultivars, although Uurainen showed a lower N uptake after anthesis than Inari and Scarlett (II). These results are in agreement with the general understanding of the role of Rubisco as a storage protein, i.e. leaves contain more Rubisco protein relative to the other leaf components and leaf senescence is a consequence of N remobilization from Rubisco (Lawlor, et al. 1989; Lemaire and Millard, 1999). The higher enzyme content of Rubisco in Scarlett leaves in both years (IV) further supports the role of Rubisco as a storage protein (Lawlor, et al. 1989; Lemaire and Millard, 1999). Eventually the other storage protein properties are met under northern conditions also, as the Rubisco protein content decreased faster than the total soluble protein content in leaves. This was demonstrated also by earlier studies (Friedrich and Huffaker, 1980; Makino et al., 1983; Millard and Catt, 1988). It seems that especially under slightly water-limiting conditions, typical for 2002, Rubisco could have played an emphasised role as a storage protein in cultivar Scarlett when compared with the old landrace Uurainen. Results from this study indicated that even though barley cultivars, especially Scarlett, demonstrated an inverse relationship between Rubisco efficiency and Rubisco content, they also had increased amounts of Rubisco.

3.5 RUE and light interception related to N use

There was no clear trend of plant breeding induced improvements in pre-heading RUE when old and modern cultivars were compared. In fact, some old barley cultivars (Urainen and Olli) tended to have higher pre-heading RUE than some of the modern barley cultivars (III), particularly at higher N supply. However, modern wheat and oat cultivars tended to have higher RUE values than old ones. Although Siddique et al. (1989) and Yunusa et al. (1993) reported that modern wheat cultivars had increased RUE values, it was also shown by Calderini et al. (1997) that some of the old Argentinean wheat cultivars were very efficient in using intercepted radiation to produce biomass when compared with some modern cultivars. This could also be the case for barley cultivars adapted to grow in the north. Maybe breeding has not modified pre-heading RUE under these conditions, as it has not changed NUE in barley cultivars. Post-heading RUE was higher than pre-heading RUE for most of the cultivars (III). This likely resulted from a high biomass accumulation during the grain filling period, since in general less than half of the total above-ground biomass was produced before heading (III). The production of the vegetative phytomass, that is, main shoot straw and tillers, continued after heading. Old and modern cultivars differed from each other only in oat, since the old cultivar Jalostettu maatiainen had higher post-heading RUE than modern cultivars. There was also a trend for old barley cultivars to have higher post-heading RUE than the modern ones (III). Green (1989) indicated that the filling grains create a demand for carbon assimilates and consequently there is increased need for senescing leaves to maintain their photosynthetic activity. Calderini et al. (1997) concluded that old and modern wheat cultivars differed in their degree of post-anthesis RUE reduction. They suggested that the capacity of the sink explains the differences between old and modern cultivars and hence, the sink strength either results in decreased or increased post-anthesis RUE. Kemanian et al. (2004) indicated that if the N needed for grain growth and packing comes from plant foliage, leaf photosynthetic capacity decreases and thereby RUE also. However, they concluded that the constant RUE values throughout the growing period could be maintained with N levels higher than just sufficient as this delays the translocation of N from the leaves. However, the most probable reason for the trend of higher post-heading RUE in old cultivars would be the reduced ability to accumulate assimilates to the grains because of low sink strength (i.e. low grain number) and therefore, they would be able to maintain sufficient N levels in leaves and also leaf photosynthetic capacity would be maintained. This is supported also by our N utilization results. Especially for oat and six-row barley, the old cultivars combined high N uptake after heading with higher biomass accumulation rate (II). Low UTE and NRE values characterizing the old oat and six-row barley cultivars also indicate the low demand for N translocation (II). When comparing the species with each other, oat and wheat had the highest increase in post-heading RUE (22% higher than pre-heading RUE), which was mostly explained by higher biomass accumulation after heading and, in the case of wheat, by the low HI. The increase in post-heading RUE in barley was lower (17%). Since RUE was especially low in the modern two-row barley cultivar Scarlett, it supports the idea of N translocation from the leaves to the grains by remobilising the stored Rubisco as described earlier (IV).

4 CONCLUSIONS

This study demonstrated that modern wheat, barley (two-row) and oat cultivars had similar NUE values under Finnish growing conditions. Thus, the first hypothesis was contradicted by the results. However, using a wider range of cultivars, the NUE comparison between the species indicated that wheat cultivars could have a comparatively lower NUE.

Modern wheat and oat cultivars had higher NUE values than did old landraces. There was a significant linear relationship between NUE and year of release for wheat and oat, but there was no clear relationship for two-row barley. However, there were also some indications that old barley cultivars differed from modern ones when rather limited cultivar selection was investigated. Thus, the second hypothesis was largely confirmed.

There was a clear relationship between UPE and NUE in all species, whereas UTE had a strong positive relationship with NUE only for oat. However, there were differences between the species in N uptake during the growing period, showing that wheat and oat differed from barley by having a higher post-heading N uptake. UTE was clearly lower in wheat than in other species. Thus, the third hypothesis was proved correct.

When further identifying the additional traits related to N translocation, our results indicated that wheat had also lower HI, NHI and NRE and therefore its N translocation efficiency was confirmed to be very low.

Old and modern cultivars did not show clear differences in pre-heading RUE, except six-row barley. Post-heading RUE differed only for oat. Hence, it can be concluded that the last hypothesis was partly contradicted by the results. Furthermore, the differences, although only moderate between cultivars for pre-heading RUE possibly indicated that old cultivars had an already relatively high biomass production ability compared with the modern ones. The difference between cultivars for post-heading RUE is most probably related to the reduced ability of old cultivars to accumulate assimilates into the grains, because of low grain number.

The described differences in N dynamics and N use efficiency shown in this work emphasise the potential and also the need for increased use of NUE characterisation traits in future in plant production research, plant breeding and in management practises. All of these research and management areas should be combined equally, in order to be able to understand and utilize for instance, the efficiency of inputs and the effects of adapted crop management practices on N use efficiency and thereby on its environmental impacts.

Based on these results, selecting for better NUE in breeding programmes would be still challenging, due to the complicity of characterising NUE. Early spring N uptake would be the way to address the better N recovery from the soil simultaneously decreasing the potential for N leaching and volatilization. During the grain filling N relocation to grains is the major part

of N dynamics and all improved N translocation traits together with increased biomass production would increase the final NUE and the quality of yield. Thus, these results may help understand the physiological differences in NUE and to identify alternative production options on spring cereals, such as the different roles species can play in crop rotations designed to meet the demands of modern agricultural practices.

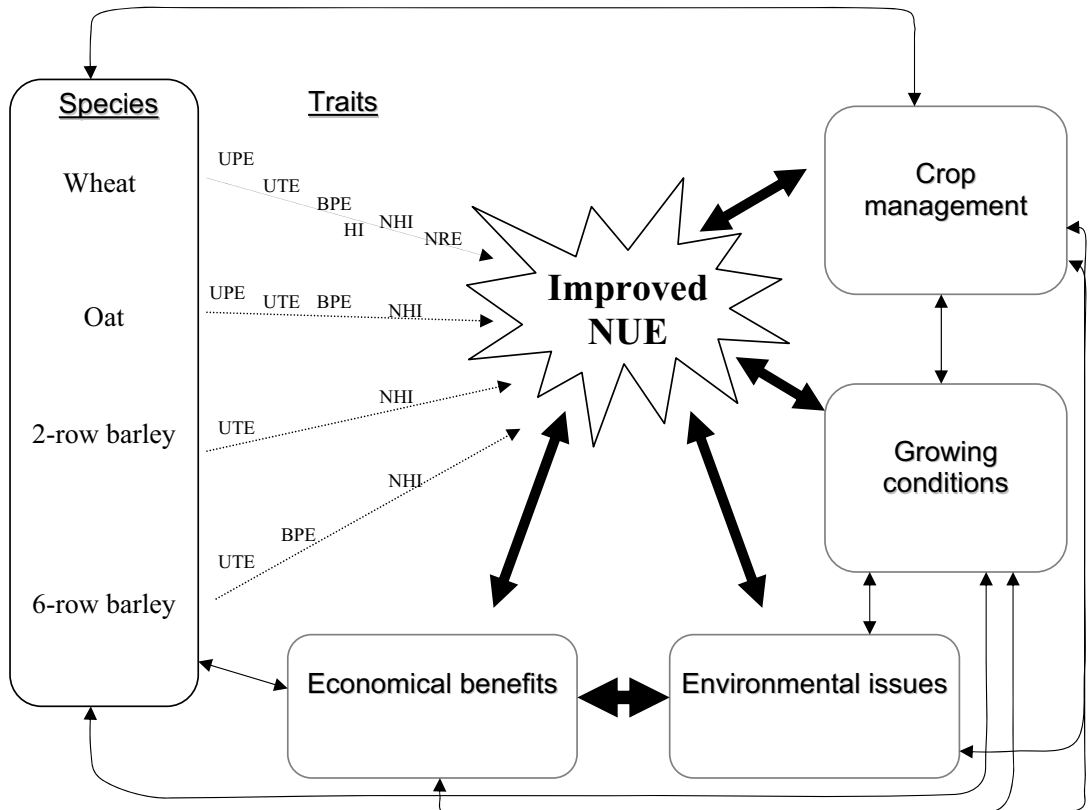


Figure 3. Interactions between species and environment affect improvement of NUE. Dependents between species and crop management influence directly farmers economical benefit as well as environmental issues. Plant production research and plant breeding influence the NUE through improvement of plant N dynamic and NUE traits through long-term selection work, whereas yearly management practises on fields affect directly to N use based to the environmental conditions.

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